IN THE SPECIFICATION

The paragraph beginning at page 1, line 3 is amended to add the section heading as follows:

FIELD OF THE INVENTION

The present invention relates to an improved method for the purification of alpha-1-acid glycoprotein, and to therapeutic uses of highly purified alpha-1-acid glycoprotein.

The paragraph beginning at page 1, line 7 is amended to add the section heading as follows:

BACKGROUND OF THE INVENTION

Alpha-1-acid glycoprotein (AAG) is a plasma glycoprotein of approximate molecular weight 41 kD. It is an acute phase protein, present in plasma at a concentration of between 0.5-1 g/l in healthy people, rising in disease states, particularly inflammatory diseases, to levels up to about 2 g/l.

The paragraph beginning at page 2, line 35 is amended to add the section heading as follows:

SUMMARY OF THE INVENTION

We have now developed a new process for removing LPS from an AAG containing preparation.

The paragraph beginning at page 3, line 8 is amended as follows:

Preferably, said resin is a particulate resin, especially an inorganic particulate resin and more preferably a hydrophilic resin. Resins with porous surfaces for example silane-based resins such as fumed silica are particularly suitable. One such fumed silica resin which may be used in the method of the invention is the commercially available fumed silica product ~~Aerosil™~~

AEROSIL™ fumed silica (Degussa AG, Frankfurt), which has siloxane and silanol groups on the surface of the particles.

The paragraph beginning at page 3, line 17 is amended as follows:

~~Aerosil™~~ AEROSIL™ fumed silica and similar resins have previously been used in the pharmaceutical industry both as a component, for example in the formulation of tablets and ointments, and also in purification processes such as the removal of lipid and lipid-like substances, and lipoprotein from plasma and plasma derived products. We are not aware of any previous suggestion to use ~~Aerosil~~ AEROSIL™ fumed silica, or any other finely divided particulate resin as a depyrogenating agent for AAG. The non-toxic nature of ~~Aerosil~~ AEROSIL™ fumed silica represents a distinct advantage over prior methods of purifying AAG which rely on separation techniques using materials which are not suitable for therapeutic applications.

The paragraph beginning at page 10, line 15 is amended to add a section title, as follows:

BRIEF DESCRIPTION OF THE DRAWINGS

The invention will now be described by way of the following non-limiting Examples, with reference to the Figures which show:

The paragraph beginning at page 10, line 25 is amended to add a section heading, as follows:

DETAILED DESCRIPTION

In the following Examples, LPS was measured by the gel clot assay for endotoxins using Limulus Amoebocyte Lysate with Atlas Bioscan *E. Coli* endotoxin as a positive control.

The paragraph beginning at page 18, line 18 is amended as follows:

EXAMPLES 6-7

Demonstration of effect of ~~Aerosil~~ AEROSIL™ fumed silica treatment upon endotoxin level

The paragraph beginning at page 18, line 27 is amended as follows:

An AAG solution at 50 g/l was prepared. This was not ~~Aerosil~~ AEROSIL™ fumed silica treated and provided a control reference sample.

The paragraph beginning at page 18, line 32 is amended as follows:

An AAG solution at 50 g/l was treated with 5% w/v ~~Aerosil 380~~ AEROSIL 380™ fumed silica and stirred at 37° C for 2 hours.

On page 19, line 1 of Table 2 is amended as follows:

TABLE 2

Example	AAG g/L	~~Aerosil~~ AEROSIL™ fumed silica % w/v	LAL Eu/ml	LAL Eu/mg
6	50	0	157	3.14
7	50	5	2.36	0.047

The paragraph beginning at page 19, line 9 is amended as follows:

The endotoxin activity of an AAG solution at 50 g/l was drastically reduced by ~~Aerosil 380~~ AEROSIL 380™ fumed silica treatment at 37° C for 2 hours.

The subheading at page 19, line 14 is amended as follows:

Optimisation of ~~Aerosil~~ AEROSIL™ fumed silica treatment concentration

The paragraph beginning at page 19, line 17 is amended as follows:

An AAG solution at 50 g/l was prepared as described in Example 1A. This was not ~~Aerosil~~ AEROSIL™ fumed silica treated and provided a control reference sample.

The paragraph beginning at page 19, line 22 is amended as follows:

An AAG solution at 50 g/l was treated with 0.1% w/v ~~Aerosil 380~~ AEROSIL 380™ fumed silica and stirred at 37° C for 2 hours.

The paragraph beginning at page 19, line 26 is amended as follows:

An AAG solution at 50 g/l was treated with 0.3% w/v ~~Aerosil 380~~ AEROSIL 380™ fumed silica and stirred at 37° C for 2 hours.

The paragraph beginning at page 19, line 30 is amended as follows:

An AAG solution at 50 g/l was treated with 0.5% w/v ~~Aerosil 380~~ AEROSIL 380™ fumed silica and stirred at 37° C for 2 hours.

The paragraph beginning at page 19, line 34 is amended as follows:

An AAG solution at 50 g/l was treated with 1% w/v ~~Aerosil 380~~ AEROSIL 380™ fumed silica and stirred at 37° C for 2 hours.

The paragraph beginning at page 19, line 38 and ending at page 20, line 1 is amended as follows:

An AAG solution at 50 g/l was treated with 3% w/v ~~Aerosil 380~~ AEROSIL 380™ fumed silica and stirred at 37° C for 2 hours.

The paragraph beginning at page 20, line 4 is amended as follows:

An AAG solution at 50 g/l was treated with 5% w/v ~~Aerosil 380~~ AEROSIL 380™ fumed silica and stirred at 37° C for 2 hours.

The heading of Table 3 is amended as follows:

TABLE 3

Example	AAG g/L	~~Aerosil~~ AEROSIL™ fumed silica % w/v	LAL EU/ml	Specific Activity EU/mg
8	50	0	25-62.5	0.5-1.25
9	50	0.1	12.5-25	0.25-0.5
10	50	0.3	6.25-12.5	0.125-0.25
11	50	0.5	7.5-12.5	0.15-0.25
12	50	1	7.5-12.5	0.15-0.25
13	50	3	3.75-7.5	0.075-0.15
14	50	5	3.75-7.5	0.075-0.15

The paragraph beginning at page 20, line 22 is amended as follows:

The clearance of endotoxin activity increased with ~~Aerosil 380~~ AEROSIL 380™ fumed silica concentration, with an optimum ~~Aerosil 380~~ AEROSIL 380™ fumed silica concentration of 3-5% w/v when treating a 50 g/l AAG solution. Optimal endotoxin clearance therefore occurs in the range of 0.6 to 1 g ~~Aerosil/g AAG~~ AEROSIL™ fumed silica/g AAG.

The subheading beginning at page 20, line 29 is amended as follows:

~~Aerosil 380~~ AEROSIL 380™ fumed silica treatment at high and low AAG concentration results in depyrogenation of a final product

The paragraph beginning at page 21, line 2 is amended as follows:

The first aliquot of AAG was concentrated from approximately 4 g/L to approximately 20 g/l by ultrafiltration using membranes with 10,000 molecular weight cut off. This aliquot was not subjected to ~~Aerosil 380~~ AEROSIL 380™ fumed silica treatment and provided a control sample.

The paragraph beginning at page 21, line 9 is amended as follows:

The second aliquot was treated with ~~Aerosil 380~~ AEROSIL 380™ fumed silica at 1 g:1 g ratio, at 37° C for 2 hours. The ~~Aerosil 380~~ AEROSIL 380™ fumed silica was removed by filtration. The AAG solution was concentrated to 20 g/l by ultrafiltration as in example 15.

The paragraph beginning at page 21, line 16 is amended as follows:

The third aliquot of AAG solution was concentrated from approximately 4 g/L to approximately 20 g/l by ultra-filtration as in example 15. The protein concentrate was ~~Aerosil 380~~ AEROSIL 380™ fumed silica treated at 1 g:1 g ratio at 37° C for 2 hours. The ~~Aerosil 380~~ AEROSIL 380™ fumed silica was removed by filtration.

The paragraph beginning at page 21, line 36 and ending on page 22, line 5 is amended as follows:

~~Aerosil 380~~ AEROSIL 380™ fumed silica has a high volume/weight ratio and can occlude a relatively large volume of aqueous solution. Treatment of a dilute protein solution with ~~Aerosil~~ AEROSIL™ fumed silica at lg:lg ration reduced the percentage w/v of ~~Aerosil~~ AEROSIL™ fumed silica. For this reason the loss of product is greatly reduced. Therefore ~~Aerosil 380~~ AEROSIL 380™ fumed silica treatment is favoured at the dilute protein stage.

The paragraph beginning at page 22, line 9 is amended as follows:

A solution of AAG produced as described in Example 1A at 50 g/L was treated with ~~Aerosil 380~~ AEROSIL 380™ fumed silica at 1 g:1 g ratio, mixing at 37° C. Samples were removed at time intervals and the ~~Aerosil 380~~ AEROSIL 380™ fumed silica removed by centrifugation. The supernatant was assayed for presence of endotoxin by LAL analysis. The results are shown in Table 5.

The paragraph beginning at page 22, line 26 is amended as follows:

Example 6 provides a control for Examples 18-22. In all cases, Examples 18-22, the endotoxin activity has been reduced. It is significant that regardless of treatment time the degree of endotoxin clearance is of the same order. The treatment time quoted in Table 5 does not include the time for ~~Aerosil 380~~ AEROSIL 380™ fumed silica removal. The treatment time of 0 minutes, has had an effective ~~Aerosil~~ AEROSIL™ fumed silica contact time of up to 20 minutes. However, optimum clearance of endotoxin occurs within this time.

The paragraph beginning at page 23, line 7 is amended as follows:

The first aliquot of AAG solution at approximately 4.5 g/l was concentrated to 100 g/L by ultrafiltration. This was not ~~Aerosil 380~~ AEROSIL 380™ fumed silica treated and provided a control sample.

The paragraph beginning at page 23, line 13 is amended as follows:

The second aliquot of AAG solution at approximately 4.5 g/l was ~~Aerosil 380~~ AEROSIL 380™ fumed silica treated at 1 g:1 g ratio at room temperature (RT), about 20° C for 2 hours. The ~~Aerosil 380~~ AEROSIL 380™ fumed silica was removed by filtration and the protein concentrated to 100 g/L by ultrafiltration.

The paragraph beginning at page 23, line 20 is amended as follows:

The third aliquot was processed as in Example 24 but the ~~Aerosil 380~~ AEROSIL 380™ fumed silica treatment temperature was 37° C.

The paragraph beginning at page 23, line 34 is amended as follows:

~~Aerosil 380~~ AEROSIL 380™ fumed silica treatment at both temperatures significantly reduced the endotoxin activity in the final product compared to the control. It appears there is no significant difference between treatment temperatures on endotoxin clearance.

The paragraph beginning at page 27, line 27 is amended as follows:

Two kits, ~~PyroBind~~ PyroBind™ (Sepracor) and END-XB15 (Atlas Bioscan Ltd.), specifically designed for endotoxin removal from aqueous protein solutions were evaluated. Both kits consist of a specific ligand, Endotoxin Neutralising Protein (ENP), coupled to rigid support. END-X B15 beads are 65 μm silica spheres coated with ENP.PyroBind™ is a hollow fibre support with coupled ENP.

The paragraphs beginning at page 28, line 12 and ending on page 29, line 6 are amended as follows:

~~PyroBind~~ PyroBind™

The manufacturers protocol was followed. An AAG solution produced as described in Example 1A at a concentration of 100 g/l was used. A second aliquot of the same solution was diluted to 20 g/l. A syringe containing 5 mls of the 20 g/l AAG was connected to one end of the hollow fibre ~~PyroBind~~ PyroBind™ unit and an empty syringe to the other end. The AAG solution was passed through the unit from one syringe to the other 5 times. 5 mls of AAG at 100 g/l was treated in the same way, but the solution was passed through the ~~PyroBind~~ PyroBind™ unit 10 times. The solutions were then subjected to LAL analysis along with non-treated control samples.

A further modified protocol was also evaluated. 6 mls of an AAG solution at a concentration of 20 g/l produced as above was subjected to 5 passes through a ~~PyroBind~~ PyroBind™ unit. 3 mls of the primary treated AAG was retained. The remaining 3 mls was subjected to a further 5 passes through a second ~~PyroBind~~ PyroBind™ unit. Both primary and secondary treated samples along with a non-treated control sample were subjected to LAL analysis.

A control experiment using Human Albumin Solution (~~Zenalb™ 4.5, BPL~~ ZENALB™ human albumin product 4.5, BioProcess Laboratories) was performed to show the ~~PyroBind~~ PyroBind™ units were active. The albumin solution was diluted to a concentration of 22.5 g/l with pyrogen free water (PFW). *Escherichia coli* control standard endotoxin #0113, PPE-E-434 (Associate of Cape Cod Inc.), was reconstituted with PFW. 0.1 ml of the endotoxin standard was added to 5 mls of albumin, mixed and passed through a ~~PyroBind~~ PyroBind™ hollow fibre unit 5 times. The treated and non treated samples were subjected to LAL analysis.

The paragraph beginning at page 29, line 13 is amended as follows:

The control albumin sample showed that the ~~PyroBind~~ PyroBind™ units successfully removed 98% of the endotoxin with 5 passes through the unit. Using the ~~PyroBind~~ PyroBind™ unit to remove endotoxin from AAG solutions showed at best only a 53% reduction in

endotoxin. Results for END-X B15 were similar to ~~PyroBind~~ PyroBind™ giving only a 59% reduction in endotoxin after 6 hours treatment. Neither of the kits met the manufacturers claims when treating AAG solutions and would not therefore be suitable for producing a clinical grade preparation.